1103-167 **Accelerated Coronary Stenosis Progression Is Associated With the ApolipoproteinC-III Content of ApoB Particles Among Those With Diabetes Mellitus**

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Background: Coronary events have been independently associated with plasma levels of apolipoprotein (apo) B-containing particles that also contain apoC-III (Lp-B:C+).This effect may be most pronounced among diabetics and may help to explain its increased vascular risk. **Methods:** Patients (n= 144) with coronary disease and low HDL-cholesterol were randomized in an angiographic trial to simvastatin plus niacin [SN(+);10–20 mg and 2–4 gm, qd], or to their placebos [SN(-)]. Mean severity of proximal stenosis, per pt, was measured at baseline and at 3-yrs follow-up. The correlation of the 3-yr change in mean stenosis severity (Δ%S) with Lp-B:C+ levels during treatment, and with Lp-B+(no C-III) levels, was computed. Adjustment was made for smoking, and baseline stenosis severity. **Results:** There were 32 men and women with diabetes (DM) or impaired fasting glucose (IFG) and 94 without DM or IFG, equally randomized to SN(+) or SN(-). Lp-B:C+ levels at baseline were 48.5 mg/dl among DM/IFG, and 46.6 among non-DM/IFG. During active therapy, Lp-B:C+ fell 32% among DM/IFG, and 25% among non-DM/IFG; Lp-B+ fell comparably in DM/IFG and in non-DM/IFG. SN(-) had minimal impact (<3% change) on these variables. Among those with DM/IFG the adjusted correlations (Spearman) between Δ%S and Lp-B:C+ was r = 0.45 (P = 0.01). Among non-DM/IFG, r = 0.04 (P = 0.71). The corresponding values for LpB+ were r = 0.34 (P = 0.05) and r = 0.25 (P < 0.01). For the tertiles of Lp-B:C+ among DM/IFG, mean Δ%S was 0.6, 2.4, and 5.1%S (P=0.03); corresponding values for non-DM/IFG were 0.9, 1.6, and 1.1 %S.
Conclusions: Levels of apoB particles lacking apoCIII were equally atherogenic among diabetics and normals; however, stenosis progression attributable to Lp-B:C+ is seen only among those with DM/IFG. Above-average levels of Lp-B:C+ may serve as markers, or as mediators, for the accelerated atherosclerosis of diabetes.

1103-170 **Enhanced Coronary Artery Disease Risk by Combining Pathogen Burden, C-Reactive Protein and Heat Shock Protein 60 Antibodies in the Absence of Heat Shock Protein 70**

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Background: We previously demonstrated that risk of coronary artery disease (CAD) was increased when pathogen burden, C-reactive protein (CRP), heat shock protein (HSP) 60 antibodies were combined, compared to considering them separately. We subsequently demonstrated that plasma levels of HSP70 (a molecule with anti-inflammatory activity) were inversely associated with CAD risk. Methods: In the present study, we analyzed the relative importance and joint effects of pathogen burden (numbers of positive serologies to cytomegalovirus, hepatitis A virus, C*hlamydia pneumoniae, Helicobacter pylori,* herpes simplex virus type 1 and type 2), CRP levels and the presence of anti-HSP60 antibodies as well as HSP70 for the risk of CAD in 421 patients (62% men, mean age 57 years). CAD was documented by angiography. Results: Traditional CAD risk factors, including age, male gender, diabetes and hypercholesterolemia, were significantly associated with CAD. High pathogen burden (>=5 antibody seropositivities), elevated CRP levels (>0.5 mg/dL) and HSP60 antibodies were also associated with, but posed a similar risk for CAD: the odds ratio (OR) with 95% CL was 1.7 (1.0-3.0) for high pathogen burden, 1.7 (1.0-3.1) for elevated CRP levels, and 1.7 (0.9-2.9) for HSP60 antibodies, respectively. However, pathogen burden combined with elevated CRP levels and HSP60 antibodies (which constituted 40% of the cohort) was more strongly associated with CAD (OR 10.6 with 95% CL 2.8-41.7). The significance persisted after adjustment for traditional risk factors. Most importantly, we found that, in the patients with high pathogen burden, elevated CRP levels and HSP60 antibodies, and who had no detectable plasma HSP70 protein (which constituted 9% of the cohort), the risk of CAD was dramatically increased. The OR of CAD reached 37.5 (4.3-330.6). Conclusion: Although confirmatory studies need to be conducted in larger cohorts, this study demonstrated that the prediction of CAD risk can be markedly increased by combining these non-traditional risk factors that reflect different processes predisposing to CAD.

1103-193 **Independent Relationship Between C-Reactive Protein and Markers of Insulin Resistance in Overweight and Obese Children**

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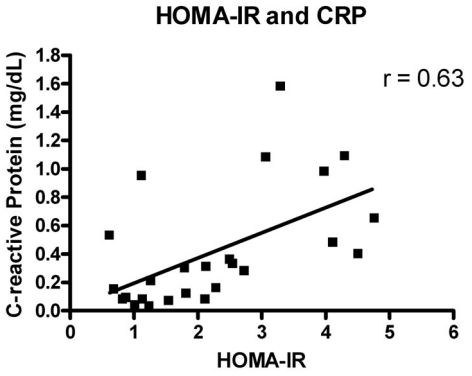
Background: In adults, systemic inflammation is associated with insulin resistance independent of obesity, however, data are lacking in children. The purpose of this study was to assess the relationship between plasma C-reactive protein (CRP) and markers of insulin resistance in overweight and obese children (BMI > 85th percentile for age/gender). **Methods:** Ultra-sensitive CRP, fasting insulin and glucose, and body composition (dual-energy x-ray absorptiometry) were assessed in 25 healthy overweight and obese children (M = 12, F = 13; age = 10.9 ± 2.0 years; BMI = 30.4 ± 6.7 kg/m^2; body fat = 44.1 ± 6.6%). The log of CRP was used for all analyses. **Results:** CRP was significantly correlated with percent body fat (r = 0.64; p = 0.001), the homeostasis model assessment for insulin resistance (HOMA-IR) (r = 0.63; p = 0.001), and fasting insulin (r = 0.62; p = 0.001) (Figure). After adjusting for percent body fat, the relationships remained significant for CRP and HOMA-IR (r = 0.49; p = 0.014), and for CRP and fasting insulin (r = 0.49; p = 0.014). **Conclusions:** 1) CRP is significantly correlated with body fatness and markers of insulin resistance in healthy overweight and obese children. 2) The association between CRP and insulin is independent of percent body fat in these individuals. These results provide evidence that in overweight and obese persons a relationship between sub-clinical inflammation and insulin resistance occurs early in life, long before the development of overt diabetes and cardiovascular disease.



1103-194 **Identification of Novel Genetic Markers Associated With Risk of Myocardial Infarction From a Genomic Scale Scan of Putative Functional Polmorphisms**

Dov Shiffman, May Luke, Olga Iakoubova, Bradley E. Aouizerat, Christian A. Zellner, Clive R. Pullinger, Denis W. Drew, Joseph J. Catanese, Diane U. Leong, Dongming Liu, Judy Z. Louie, David Lew, Carmen H. Tong, David A. Ross, Linda B. McAllister, Charles M. Rowland, Kit F. Lau, James J. Devlin, Mary J. Malloy, John P. Kane, University of California, San Francisco, San Francisco, CA, Celera Diagnostics, Inc., Alameda, CA

Background: Myocardial infarction (MI) is a multi-factorial disease associated with both environmental and genetic factors. Identification of genetic polymorphisms associated with increased risk of MI could lead to prediction of risk and provide a mechanistic basis for individualized therapeutic intervention. Methods: We set out to identify novel single nucleotide polymorphisms (SNPs) that are associated with risk of MI. We tested a comprehensive set of 16,000 SNPs selected for their putative functional properties. The majority of the SNPs we tested (~80%) are exonic putative functional SNPs, i.e. missense, nonsense and splice donor/acceptor polymorphisms. The rest were selected for their potential effect on transcription and mRNA stability. Results: Allele frequencies in 340 male MI cases and 503 male controls were determined for all 16,000 SNPs by a PCR based methodology. To increase the speed and capacity of the screen, allele frequencies were measured in pooled samples. Pools of DNAs from 50 individuals with similar phenotypes were generated. This small pool size enabled stratified analysis of the data. A thousand markers were selected for further study based on their effect size, significance level, and the presence of significantly associated neighboring SNPs. To date, eighty markers were retested in a second sample set. Conclusions: We have validated the association of MI with three previously reported genes (e.g. PON1, P-Selectin and ICAM-1). We also have independently replicated association of missense SNPs in three novel genes (an immune cell receptor on chromosome 20p, a zinc finger protein on 3q and a WD repeat protein on 2q) not previously reported to be associated with MI. Two additional SNPs are located in predicted but uncharacterized genes on chromosomes 1 and 3. The p values of the replicated markers range from 0.04 to 0.004, and their odds ratios range from 1.3 to 1.7. The moderate effect size of these markers and their relatively high risk allele frequencies (12 to 95 percent) are consistent with the common disease - common variant hypothesis.

1103-195 **Genetic Predictive Factors for Restenosis in Diabetic Patients After Percutaneous Transluminal Coronary Angioplasty**

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Background: Patients with diabetes mellitus have a worse clinical outcome after percutaneous transluminal coronary angioplasty (PTCA) in comparison to non-diabetic patients. Different mechanisms are thought to play a role in the pronounced neointimal formation, which is probably the main process of restenosis in diabetic patients. Genetic epidemiology might provide more insights into these mechanisms. Furthermore, stratification according to the genetic make-up will enable tailoring of interventional treatment to the individual patient. The aim of this study was to evaluate if various gene polymorphisms can predict clinically important restenosis after PTCA in patients with diabetes mellitus.
Methods: The GEnetic DEterminants of Restenosis (GENDER) project was a multicenter prospective cohort study, which included 3,146 patients after successful PTCA of which 459 (14.6%) were diabetics. Six patients were excluded from follow-up because of an event in the first 30 days. Genotyping in these patients was performed for different polymorphisms in several candidate genes.
Results: A total of 453 diabetic patients, with a mean age of 64.01±10.47 were followed. Of these patients 150 (33.1%) were insulin dependent, stenting was performed in 317 (70.0%) patients and most patients were treated for stable angina (314, 69.3%). The pri-

mary endpoint of target vessel revascularization (TVR) was reached in 63 (13.9%) patients. Several polymorphisms seem to play a role in the restenotic process. So far we identified an association between interleukin 4 (p=0.026) and three polymorphisms in the adrenergic beta-2 receptor (p=0.064,0.011,0.077) and TVR. Also cytotoxic T-lymphocyte-associated protein 4 seems to be associated with TVR (p=0.032) More genotyping is being performed at this moment.

Conclusion: Several polymorphisms seem to play a role in the restenotic process in patients with diabetes mellitus. Interleukin 4, cytotoxic T-lymphocyte-associated protein 4 and adrenergic beta-2 receptor seem to be three important genes in the process of restenosis in diabetics. This can lead to a better risk stratification and to a more tailored therapy for diabetic patients to prevent restenosis after PTCA.

1103-196 Circulating Endothelial Progenitor Cells in Patients With Unstable Angina: Association With Systemic Inflammation

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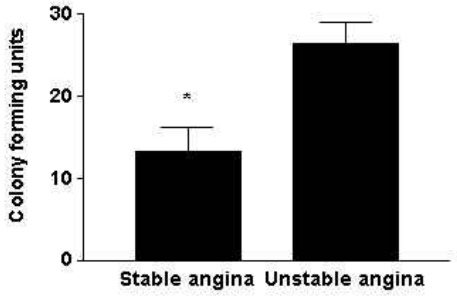
Background: Endothelial progenitor cells (EPC) are present in the peripheral circulation and can develop a functional endothelial phenotype. Number and function of circulating EPC is altered in atherosclerosis, diabetes and after myocardial infarction. We studied the number (num) and adhesive properties of EPC from patients (pts) with unstable-angina and no evidence of cardiac necrosis.

Methods: Pts with either unstable-angina (UA) (n=29) and no cardiac necrosis, and pts with stable-angina (SA) (n=12) with similar atherosclerotic risk factors, medication use, and coronary vessel disease. The number of circulating EPC was determined by colony-forming unit assay and their adhesive properties were determined by their capacity to bind immobilized-fibronectin. High-sensitivity C-reactive protein (CRP) was determined in all pts.

Results: Circulating EPC were significantly increased in patients with UA as compared with SA (24.5±2.6 versus 13.3±2.9). 7 pts with UA followed for 3 months after clinical stabilization exhibited a near 50% reduction in num of circulating EPC. Adhesive capacity of EPC from UA and SA did not differ. A positive correlation was found between systemic CRP levels and circulating EPC num but not with their adhesive capacity.

Conclusion: Pts with UA and no evidence of cardiac necrosis exhibit increased circulating EPC. System inflammation, in addition to recognized growth factors, could play a role in peripheral mobilization of EPC in patients with anginal-syndromes.



1103-197 Chlamydia Pneumoniae-Heat Shock Protein 60 Induces a Highly Specific Persistent Immune Response in Patients With Unstable Angina Independently From IgG and IgA Seropositivity

Domenico Cianflone, Alessandra Ciervo, Giulia Angeloni, Marco Magnoni, Claudia Monaco, Gaetano Lanza, Antonio Rebuzzi, Germano Melissano, Roberto Chiesa, Filippo Crea, Antonio Cassone, Attilio Maseri, Francesco Marchi, Giuseppe Ciriello, Nadia Aspromonte, Cesare Volterrani, Paolo Spallarossa, Gianni Destro, Raffaele Bugiardini, Massimo Ciavolella, Gianpietro Leone, Livio Dei Cas, The SPAI Study Investigators, Università Vita Salute San Raffaele, Milano, Italy, Istituto Superiore di Sanità, Roma, Italy

Background: The humoral response against *Chlamydia Pneumoniae* (CP)-HSP60, an highly immunogenic molecule with high degree of sequence homology with the human HSP60, was found to be highly specific for acute coronary syndromes.

Methods: Among 984 patients with unstable angina (UA) included in the SPAI (Stratificazione Prognostica Angina Instabile) Study, we selected 119 subjects with unfavourable and 119 with good outcome (as combined end point: death, myocardial infarction and readmission for UA within 180 days after admission) matched for age, gender, risk factors and clinical presentation. As stable atherosclerotic controls (PVD) we assessed 66 patients scheduled for carotid endarterectomy without evidence of coronary disease (excluded by dobutamine stress echocardiography). In all patients we analized Cp-HSP60 IgG, measured by an in-house ELISA, Cp-IgG and CP-IgA levels, measured by a commercially available microimmunofluorescence assay (Labsystems), with titers $\geq$ 1:32 regarded as positive. In UA population we also assessed the time course of seropositivity (defined as an ELISA reading of > 0.30) at discharge and at 90 days.

Results: In UA, IgG and IgA CP-seropositivity was only found in 58% (vs PVD 80.3% p<0.01) and 21% (vs PVD 30.3%; ns) respectively, but all UA patients on admission were positive (100%) for antibodies against CP-HSP60, conversely only 12% of PVD were positive for antibodies against CP-HSP60.

In UA CP-HSP60 antibody titers on admission were not correlated to clinical presentation, outcome, C-reactive protein or Troponin I levels.

In UA, CP-HSP60 antibody titers increased significantly at 90 days [median (range); admission 0.50 (0.3-1.0) vs discharge 0.60 (0.3-1.3) respectively; p < 0.01].

Finally, CP-HSP60 titers in UA were higher in CP-seropositive than seronegative patients, while no difference was observed in PVD.

Conclusion: Seropositivity to CP-HSP60 is highly specific for UA and increases significantly up to 90 days, despite stability of IgG and IgA antibody titres against CP, most likely suggesting an antigenic mimicry.

POSTER SESSION

1104

Therapeutic Angiogenesis

Monday, March 08, 2004, 3:00 p.m.-5:00 p.m.
Morial Convention Center, Hall G
Presentation Hour: 3:00 p.m.-4:00 p.m.

1104-189 Enhancement of Bone Marrow-Derived Endothelial Progenitor Cell Differentiation by Protease-Activated Receptor-1 Activation

Sima T. Tarzami, Guoming Wang, Lisa Green, JaiPal Singh, Eli Lilly and Company, Indianapolis, IN

Background: Recent advances in stem cell research have revealed that endothelial progenitor cells (EPCs) from bone marrow play important role in angiogenesis in response to injury and ischemia. The specific mediators involved in the mobilization, recruitment, differentiation and incorporation of EPCs into the blood vessels are not understood. Since coagulation factors play important role in injury response and angiogenesis, we have investigated the role of protease-activated receptor (PAR) in EPC differentiation and proliferation.

Methods: Mouse bone marrow cells were isolated and plated on fibronectin-coated dishes for 10-14 days. Later, adherent cells were treated with thrombin or the indicated agent for 4 days and then analyzed by fluorescence activated cell sorter (FACS). FACS analysis was performed to determine the effect of thrombin on other progenitor cell markers such as CD34, c-kit, AC133 and VE-cadherin. Cell prolifiration was assayed by measuring DNA synthesis and it was determined by measurement of ^{3}[H] thymidine uptake. Cells morphology was examined using confocal microspy.

Results: Treatment of CD34^{+} cells with thrombin or PAR-1 activating-peptide produced a significant increase in the number of VE-cadherin positive cells. Hirudin or a small molecular weight inhibitor of thrombin attenuated the increase in VE-cadherin positive cells. Blocking the vascular endothelial growth factor (VEGF) receptor 1 or VEGF receptor 2 using neutralizing antibodies did not produce a significant change in EPC differentiation in response to thrombin.

Conclusion: These result, for the first time, show a direct role of thrombin and PAR-1 in EPC proliferation and differentiation. These findings also suggest that thrombin may contribute to vascular regeneration by modulating endothelial progenitor cells.

1104-190 Angiogenic Profiling of the Ischemic Human Heart In Vivo

Wang Yongzhong, Anders Gabrielsen, Patrick Lawler, Gabrielle Berne Paulsson, Daniel Steinbruchel, Goran Hansson, Jens Kastrup, Karolinska Institute, Stockholm, Sweden, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark

Background: At present, the understanding of endogenous expression of factors involved in angiogenesis in the myocardium is incomplete. Therefore, we performed Affymetrix® GeneChip analysis on normal and chronic ischemic myocardium before and during acute ischemia and reperfusion stress in humans.

Methods: Myocardial biopsies were sampled at baseline, after 45 min of acute total ischemia, and after 30 min of reperfusion recovery from both normally perfused and chronic reversibly ischemic myocardium in 6 patients undergoing coronary artery by-pass grafting. RNA was isolated, amplified with established techniques, and hybridized to HG_U133A Affymetrix® Genechip arrays. A total of 33 arrays were performed, normalized with the RMA algorithm and analyzed for expression patterns of pro- and anti-angiogenic factors.

Results: Array analysis demonstrated increased baseline expression of VEGF-C and CYR61 angiogenic inducer in chronic ischemic myocardium compared with healthy tissue. VEGF-A expression did not differ between chronic ischemic and normal myocardium, and was induced in response to ischemia in both tissues. Interestingly, expression of several potent anti-angiogenic factors, i.e. thrombospondins and endostatin tended to increase in response to 45 min of acute ischemia in the diseased, ischemic tissue, but not in normally perfused myocardium. Furthermore, chronic ischemic tissue exhibited a fibrotic response to acute ischemia and reperfusion (e.g. increased collagen and fibronectin expression), whereas normal myocardium did not.

Conclusion: These findings suggest that, on a transcriptional level, the balance between angiogenesis and fibrosis in the myocardium differs between healthy and chronic ischemic tissue. Although chronic ischemic myocardium expresses pro-angiogenic molecules, this expression seems to be more strongly opposed by expression of anti-angiogenic factors and super-imposed by a fibrotic response. These features should be kept in mind when designing therapeutic angiogenic treatment.